

# **Antibiotics in the Environment: Sources, Fate, Exposure, and Risk**

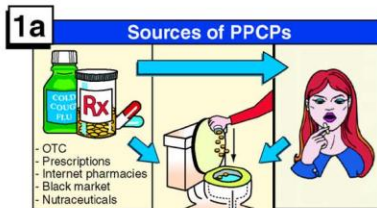
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# Outline of Presentation

- Conceptual model of the sources and fate of antibiotics and potential **non-AMR** risk
- Provide some detail of fate processes in water, soil, waste and water treatment
- Present data on antibiotics in soil and water
- Perform simple screening assessment for non-resistance risk to humans & ecosystem
- New research on measuring chronic exposure to antibiotics in water

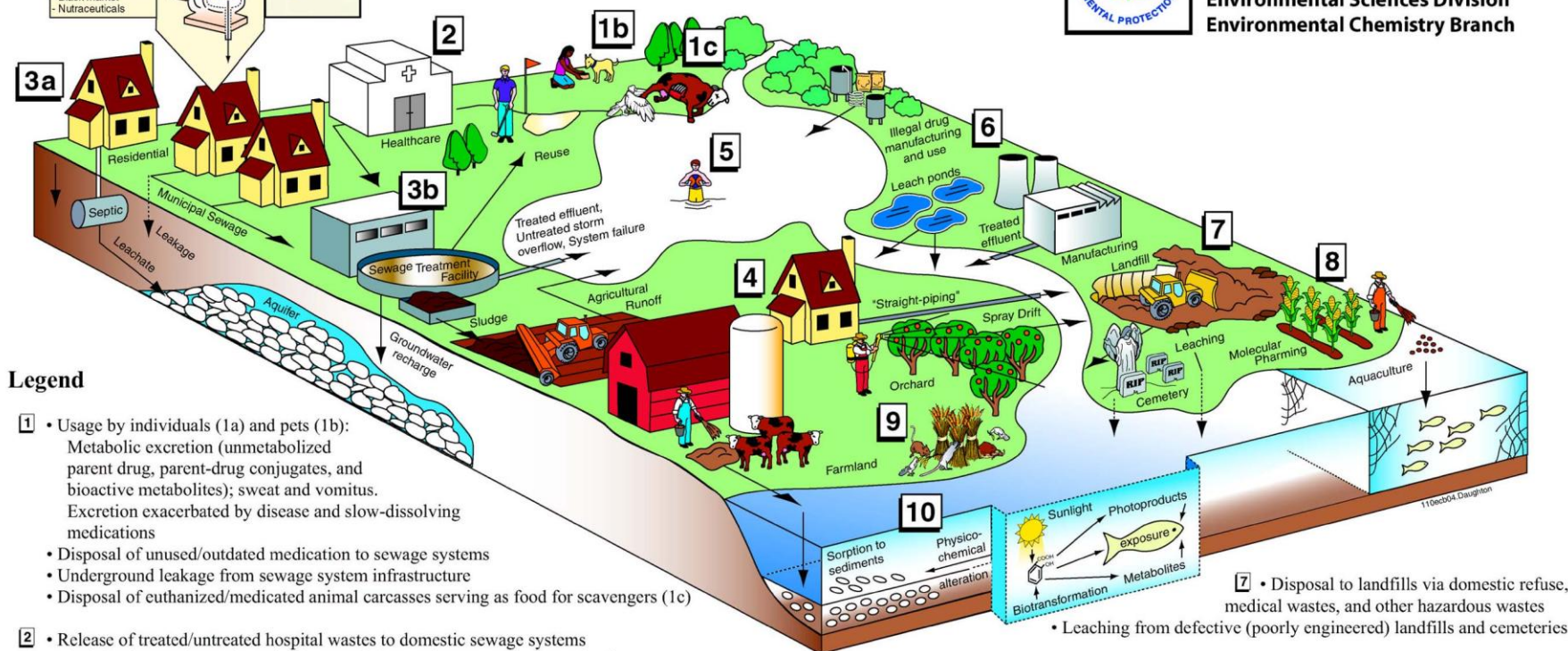


# Origins and Fate of PPCPs<sup>†</sup> in the Environment

<sup>†</sup>Pharmaceuticals and Personal Care Products

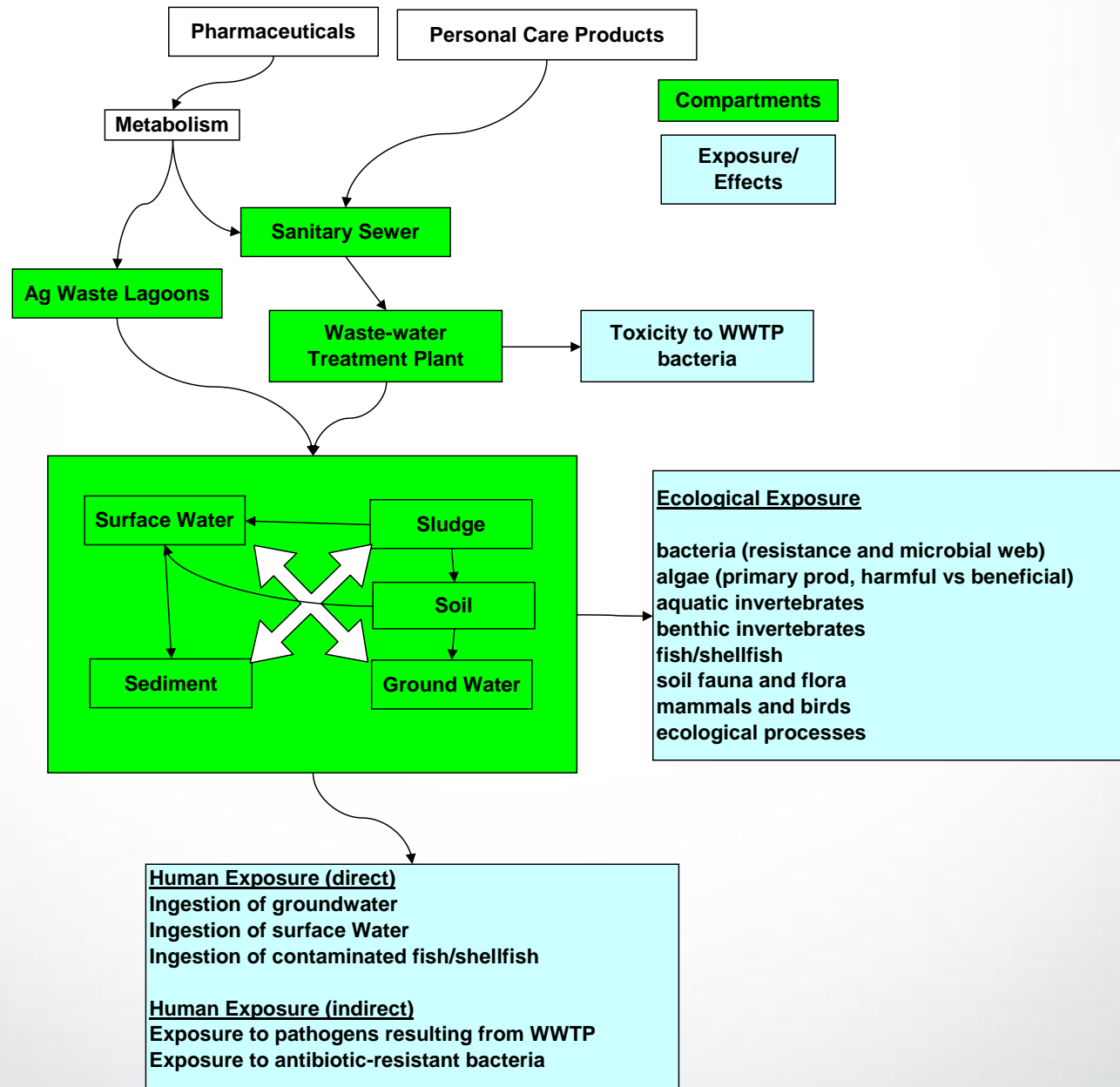


U.S. Environmental Protection Agency  
Office of Research and Development  
National Exposure Research Laboratory  
Environmental Sciences Division  
Environmental Chemistry Branch

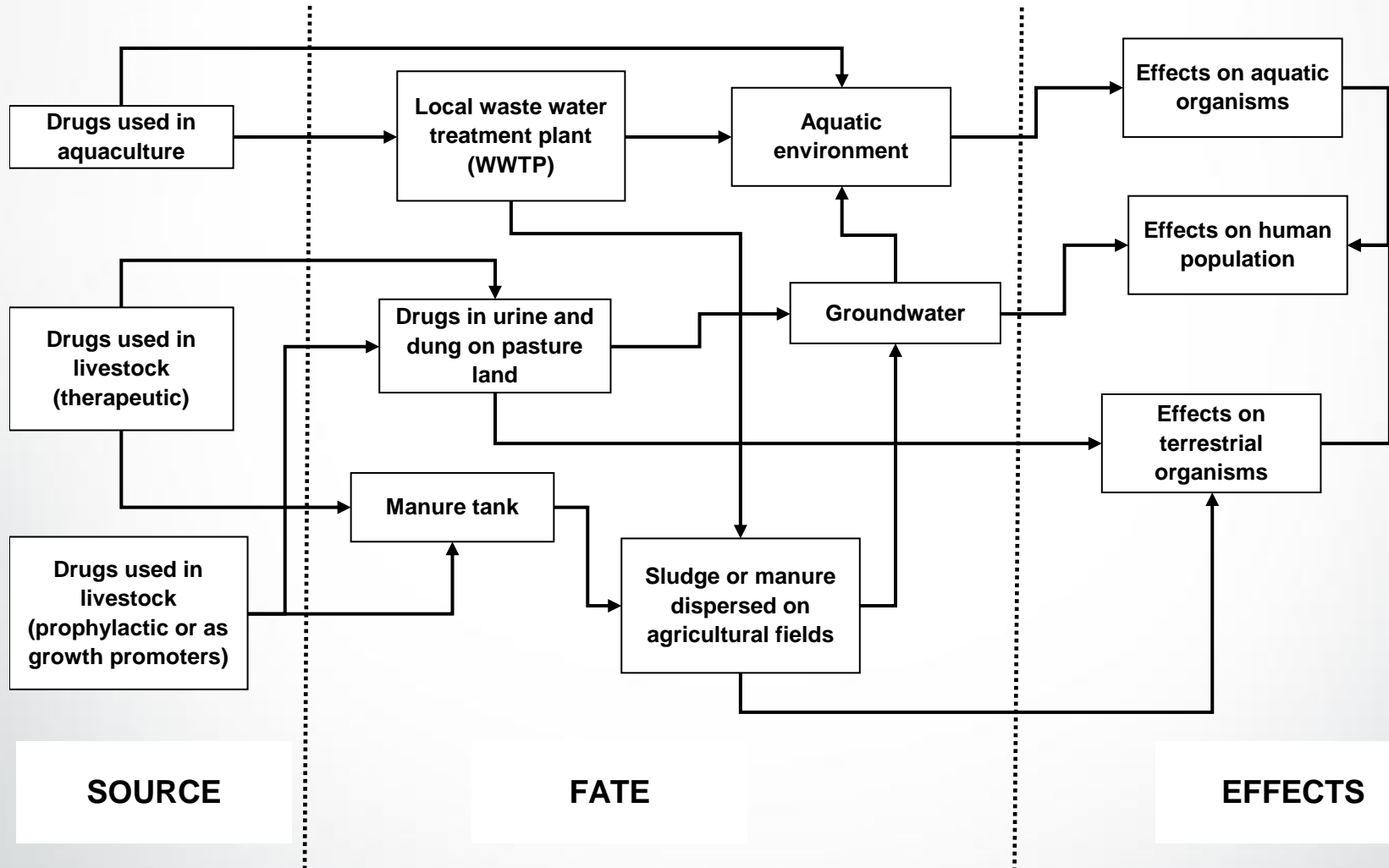


## Legend

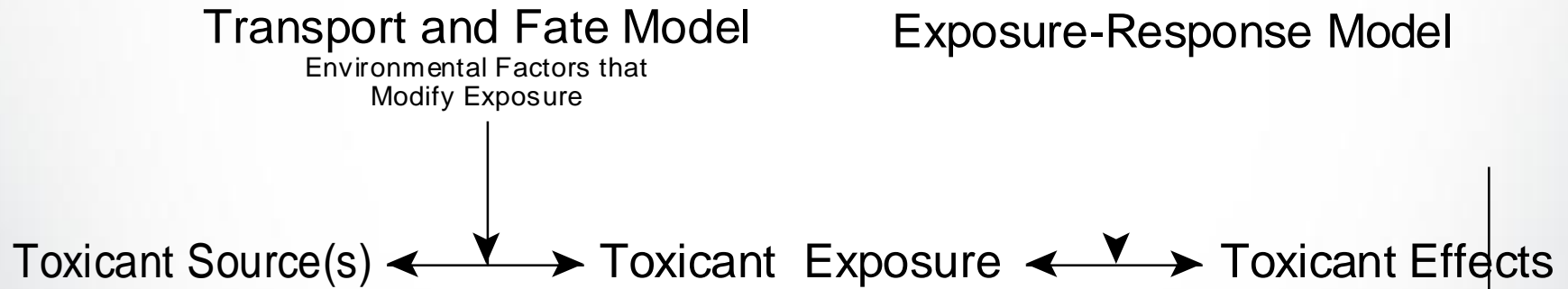
- Use by individuals (1a) and pets (1b):  
Metabolic excretion (unmetabolized parent drug, parent-drug conjugates, and bioactive metabolites); sweat and vomitus.  
Excretion exacerbated by disease and slow-dissolving medications  
• Disposal of unused/outdated medication to sewage systems  
• Underground leakage from sewage system infrastructure  
• Disposal of euthanized/medicated animal carcasses serving as food for scavengers (1c)
- Release of treated/untreated hospital wastes to domestic sewage systems (weighted toward acutely toxic drugs and diagnostic agents, as opposed to long-term medications); also disposal by pharmacies, physicians, humanitarian drug surplus
- Release to private septic/leach fields  
• Treated effluent from domestic sewage treatment plants discharged to surface waters or re-injected into aquifers (recharge)  
• Overflow of untreated sewage from storm events and system failures directly to surface waters
- Transfer of sewage solids ("biosolids") to land (e.g., soil amendment/fertilization)  
• "Straight-piping" from homes (untreated sewage discharged directly to surface waters)  
• Release from agriculture: spray drift from tree crops (e.g., antibiotics)  
• Dung from medicated domestic animals (e.g., feed) - CAFOs (confined animal feeding operations)
- Direct release to open waters via washing/bathing/swimming
- Discharge of regulated/controlled industrial manufacturing waste streams  
• Disposal/release from clandestine drug labs and illicit drug usage
- Disposal to landfills via domestic refuse, medical wastes, and other hazardous wastes  
• Leaching from defective (poorly engineered) landfills and cemeteries
- Release to open waters from aquaculture (medicated feed and resulting excreta)  
• Future potential for release from molecular pharming (production of therapeutics in crops)
- Release of drugs that serve double duty as pest control agents:  
examples: 4-aminopyridine, experimental multiple sclerosis drug → used as avicide; warfarin, anticoagulant → rat poison; azacholesterol, antilipidemics → avian/rodent reproductive inhibitors; certain antibiotics → used for orchard pathogens; acetaminophen, analgesic → brown tree snake control; caffeine, stimulant → *coqui* frog control
- Ultimate environmental transport/fate:  
• most PPCPs eventually transported from terrestrial domain to aqueous domain  
• phototransformation (both direct and indirect reactions via UV light)  
• physicochemical alteration, degradation, and ultimate mineralization  
• volatilization (mainly certain anesthetics, fragrances)  
• some uptake by plants  
• respirable particulates containing sorbed drugs (e.g., medicated-feed dusts)



## Veterinary Pharmaceuticals



## Prospective and Retrospective Assessments



How does the environment change effective exposure to antibiotics?

Can we measure it?

Can we model it?



# Antibiotics in Environmental Media

In 2000, roughly 17,000 tons of antibiotics were produced in the US

~70% used on livestock farming

Antibiotics detected in

waste (10-12,000  $\mu\text{g/kg}$ )

soil (0-200  $\mu\text{g/kg}$ )

sediment (0-25  $\mu\text{g/kg}$ )

(500-4000  $\mu\text{g/kg}$  aquaculture)

ground water (0-400 ng/L)

surface water (0-1,900 ng/L)

drinking water (0-200 ng/L)

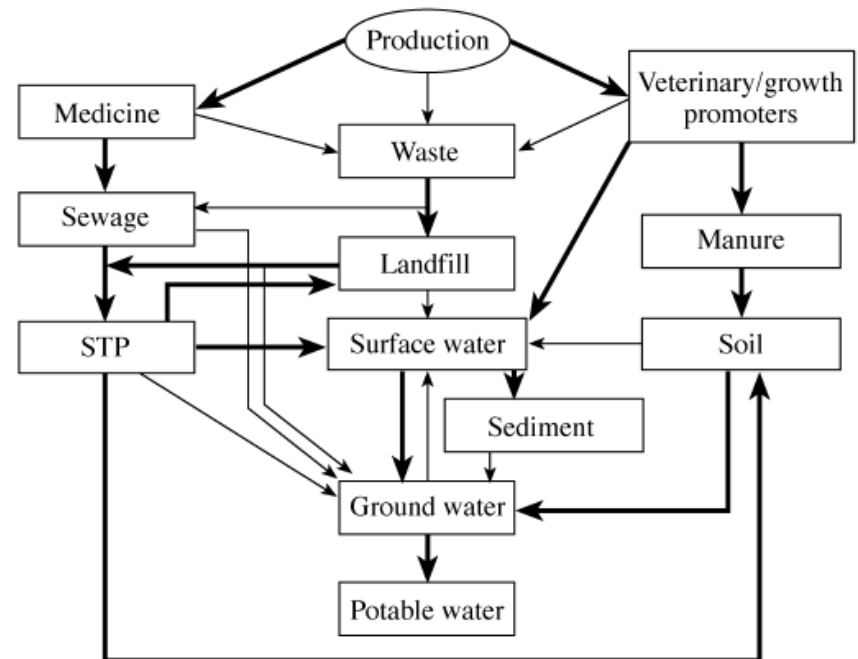


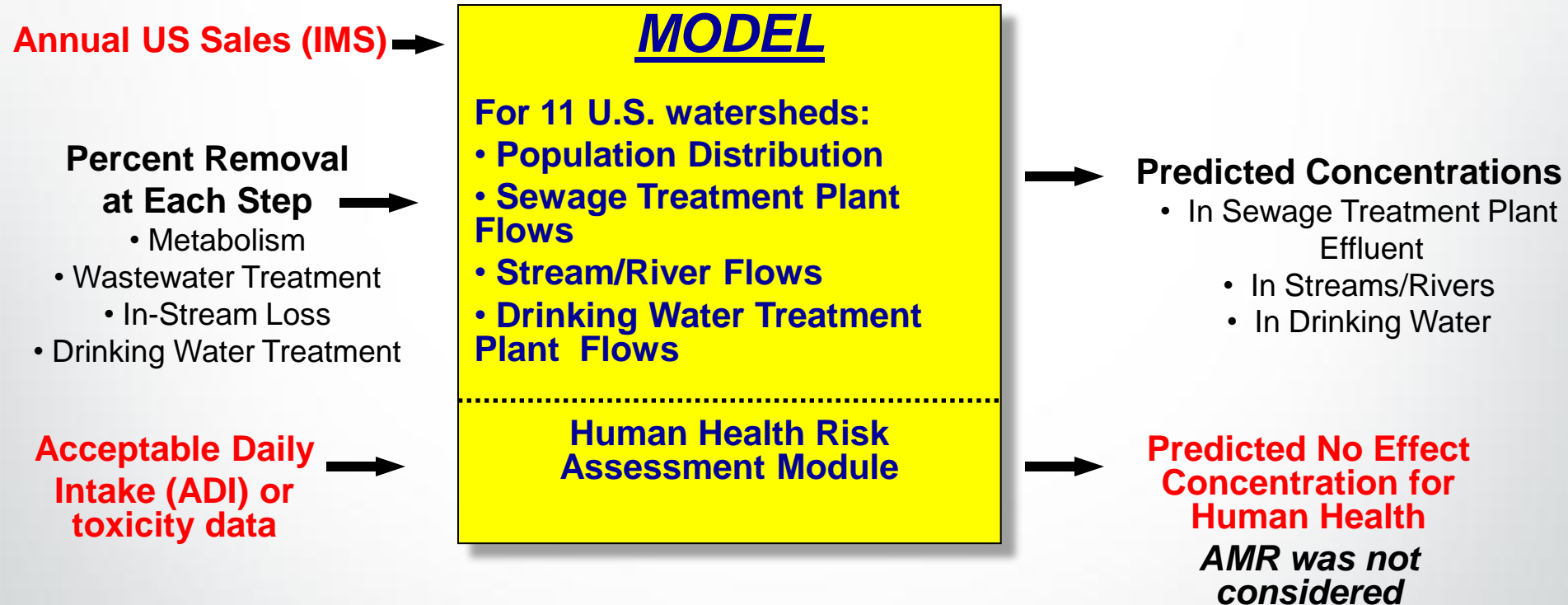
Figure 1. Sources and distribution of pharmaceuticals in the environment<sup>1</sup> (STP: sewage treatment plant).

# The PhATE™ Model

## (Pharmaceutical Assessment and Transport Evaluation)

### INPUTS

### OUTPUTS





# Summary of PhATE Screening Study

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- Anderson, P. D., et al. (2004) Screening Analysis of Human Pharmaceutical Compounds in U.S. Surface Waters, *Envir. Sci. Tech.*, 38:838-849
- PhATE PECs (Predicted) vs. USGS MECs (Measured) for 11 compounds:
  - PEC/MEC in agreement for 2;
  - $PEC < LOD$  (Limits of Detection) for 3; evaluate potential effects below LOD;
  - $PEC > MEC$  for 3; Depletion unaccounted for by model, evaluate impact of POTW and in-stream removal;
  - $PEC \ll MECs$  for 3; Comparing the PECs to the measured data identified some questionable analytical findings.

# Estimating Exposure

- **Persistence**
  - Rate constants are dependent on many environmental parameters due to multiple mechanisms of degradation (hydrolysis, photolysis, biotic, de-conjugation)
  - Overall half-lives range from many hours to many months, often with large uncertainty
- **Partitioning (between water and particles)**
  - Multiple mechanisms of sorption to soil/sediment
  - Dependent on many parameters (pH, CEC, metals)
  - Bioaccumulation potential is generally low

## Simplified model of the partitioning processes of chemicals

P: Parent compound

M: metabolites

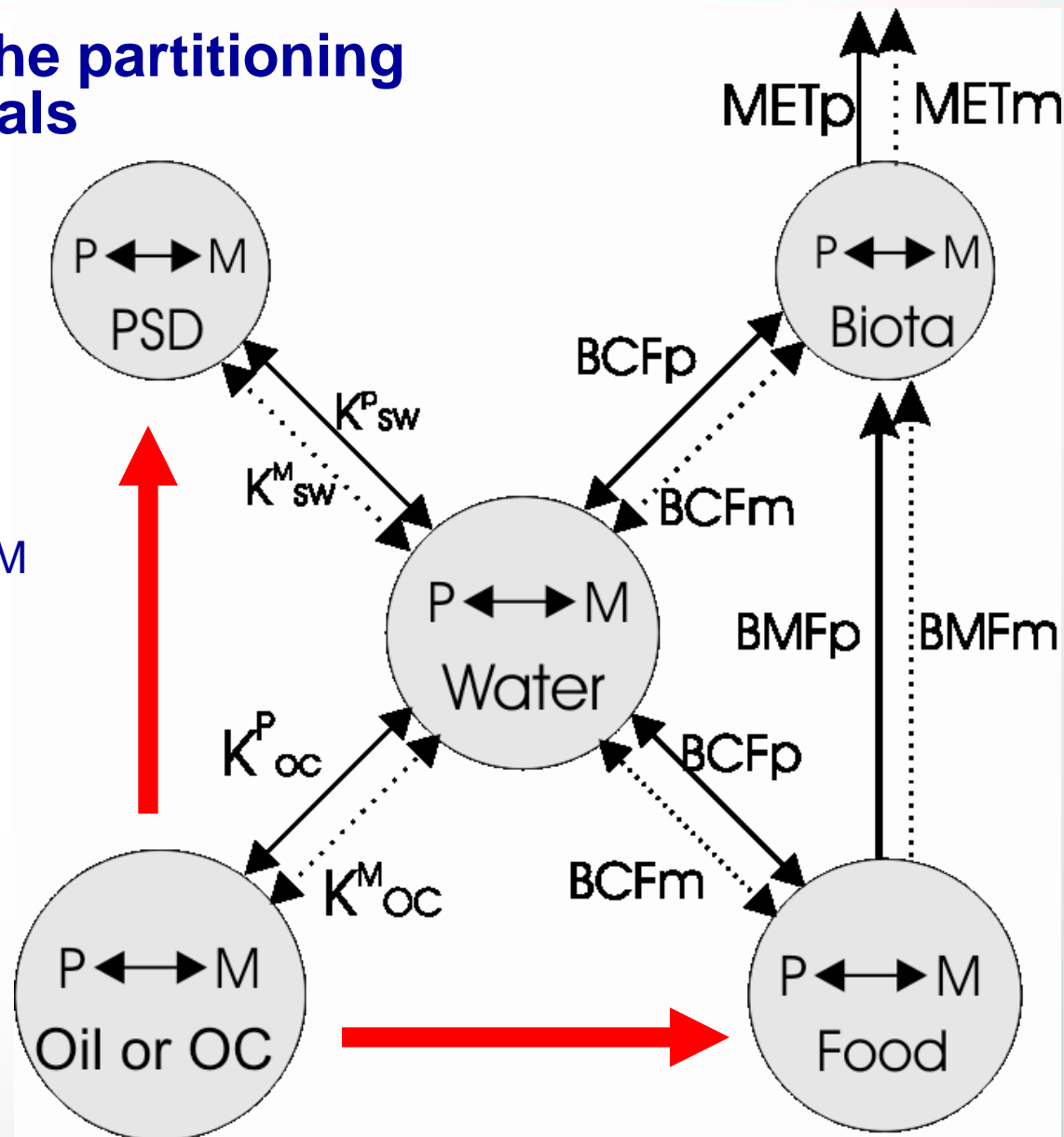
$K_{PSW}$  and  $K_{MSW}$ : PSD-water partition coefficients of P and M

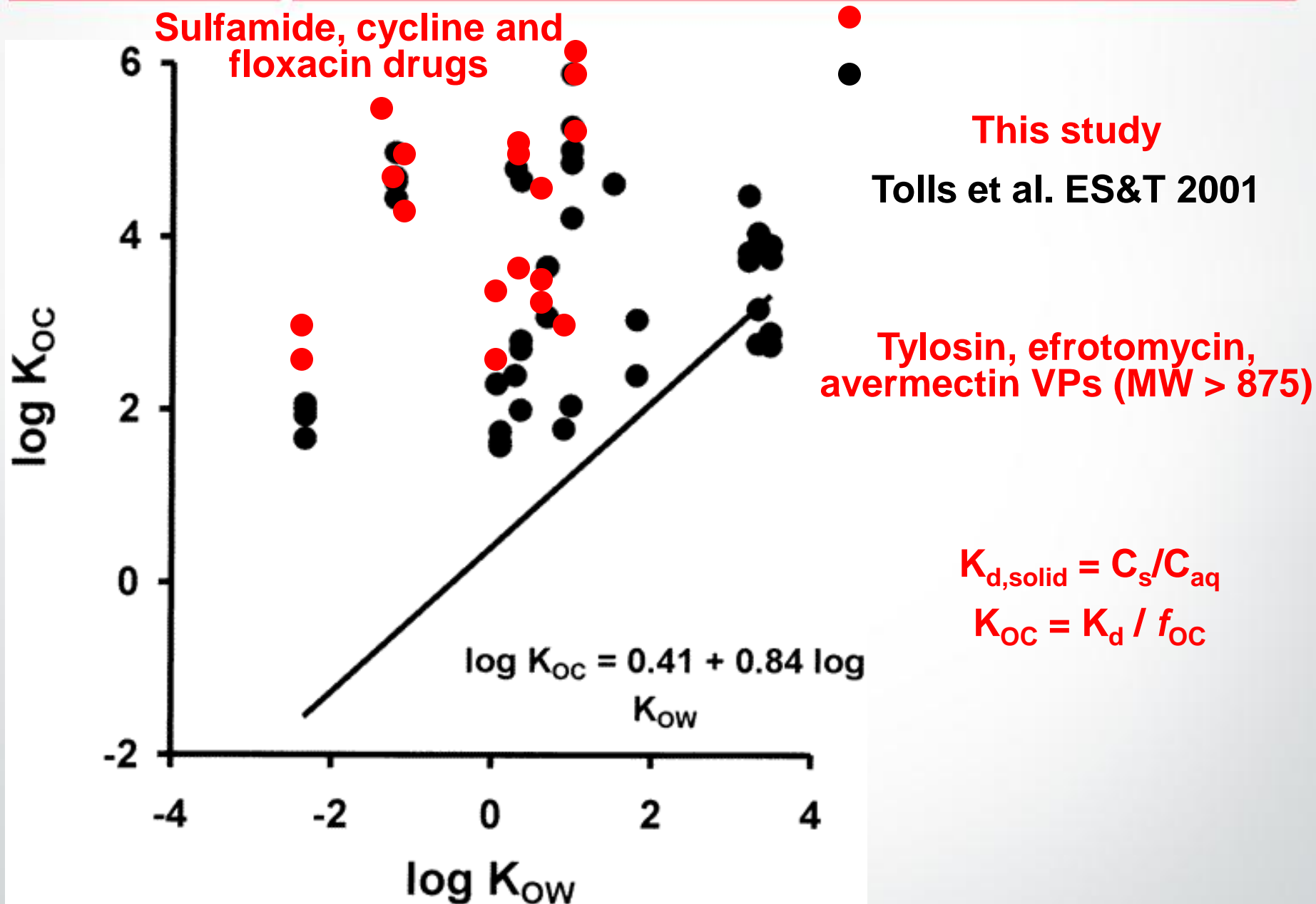
$K_{POC}$  and  $K_{MOC}$ : oil or other organic carbon sorption coefficients of P and M

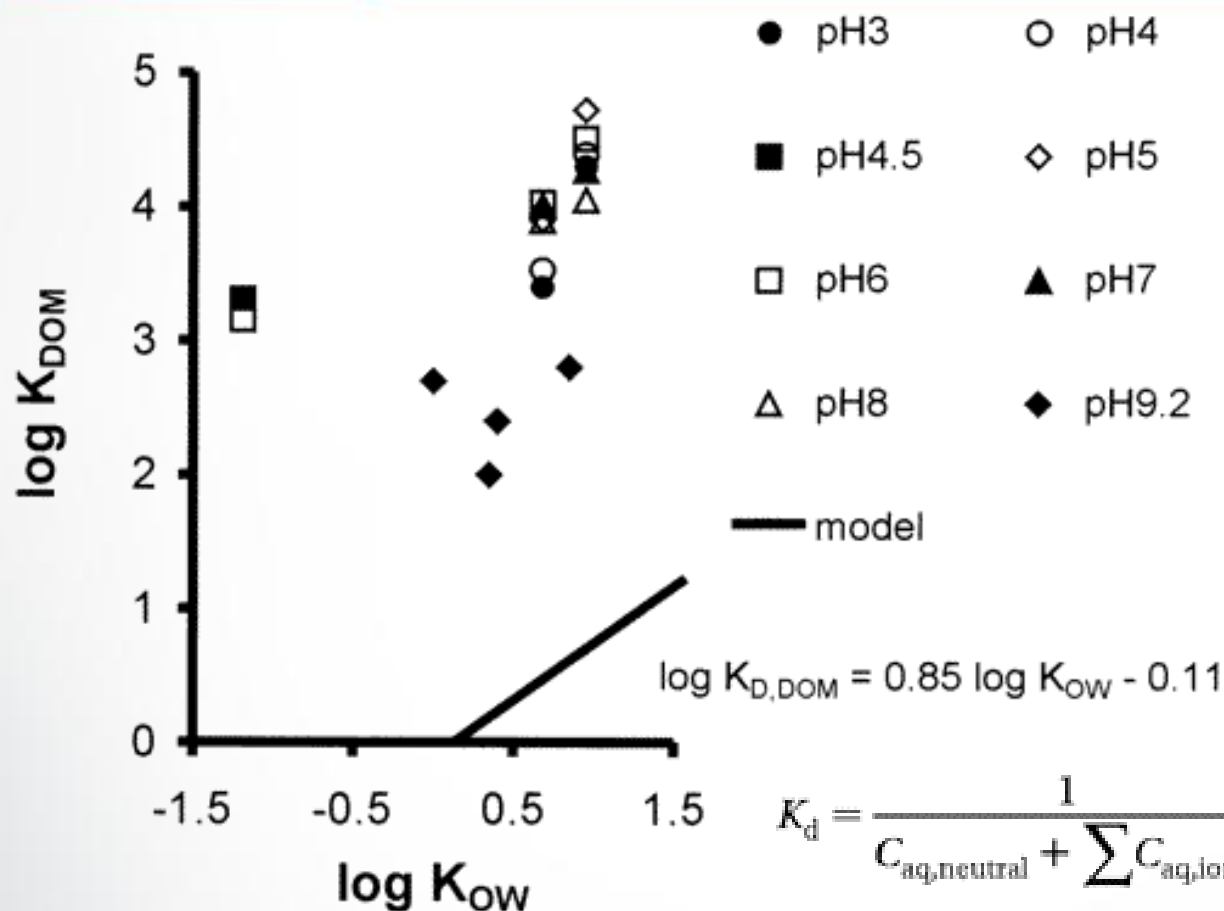
BCF: bioconcentration factor

BMF: biomagnification factor

MET: metabolic clearance







$$K_d = \frac{1}{C_{aq,neutral} + \sum C_{aq,ionic}} (C_{OM}f_{OM} + C_{min}A_{min} + C_{ic}\sigma_{ic}A_{ic} + C_{rxn}\sigma_{rxn}A_{rxn}) \quad (4)$$

Plot of the  $\log K_{d,DOM}$  data against hydrophobicity expressed as  $\log K_{ow}$ . The solid line is a regression line obtained for a wide range of neutral organic chemicals.  $K_{d,DOM}$  must be expressed as a combination of all important sorption mechanisms.

# Partitioning

- Sorption
  - Adsorption
  - Absorption
- Partitioning
  - $K_{OC} = f_{OC} * K_D$

Available literature values for partitioning coefficients of selected VAs in various environmental matrices

Compound (s)	Matrices	pH	OC (%)	$K_d$ (l kg <sup>-1</sup> )	$K_{oc}$ (l kg <sup>-1</sup> )	References
Sulfachloropyridazine	Clay loam, sandy loam	6.5–6.8	NR	0.9–1.8		Boxall et al. (2002)
Sulfadimidine	Sand, loamy sand, sandy loam	5.2–6.9	0.9–2.3	0.9–3.5	80–170	Langhammer and Buening-Pfaue (1989)
Sulfamethazine	Sand, loamy sand, sandy loam	5.2–6.9	0.9–2.3	0.6–3.2	82–208	Langhammer (1989)
Sulfapyridine	Silty loam	6.9–7.0	1.6–2.4	1.6–7.4	101–308	Thiele (2000)
Sulfanilamide	Whole soil, clay, sand fraction	6.7–7.0	1.6–4.4	1.5–1.7	34–106	Thiele-Bruhn et al. (2004)
Sulfadimidine	Whole soil, clay, sand fraction	6.7–7.0	1.6–4.4	2.4–2.7	61.0–150	Thiele-Bruhn et al. (2004)
Sulfadiazine	Whole soil, clay, sand fraction	6.7–7.0	1.6–4.4	1.4–2.8	37–125	Thiele-Bruhn et al. (2004)
Sulfadimethoxine	Whole soil, clay, sand fraction	6.7–7.0	1.6–4.4	2.3–4.6	89–144	Thiele-Bruhn et al. (2004)
Sulfapyridine	Whole soil, clay, sand fraction	6.7–7.0	1.6–4.4	3.1–3.5	80–218	Thiele-Bruhn et al. (2004)
Sulfathiazole	Topeka clay loam	NR	1.0	0.6	NR	Thurman and Lindsey (2000)
Tylosin	Loamy sand, sand	5.6–6.3	1.1–1.6	8.3–128	553–7990	Rabolle and Spiid (2000)
	Silty clay, clay, sand	5.5–7.4	0.4–2.9	5.4–6690	1350–95532	Sassman et al. (2003)
Tylosin A-aldol	Silty clay, clay, sand	5.5–7.4	0.4–2.9	516–7740	1290–266896	Sassman et al. (2003)
Tylosin	Pig manure	NR	NR	45.5/270	110	Loke et al. (2002)
Tylosin	Clay loam, sandy loam	NR	2.2–4.4	66–92	NR	Gupta et al. (2003)
	Pig manure	9.0 <sup>a</sup>	0.13–0.16	38.6–107.5	241–831	Kolz et al. (2005a)
Oxytetracycline	Loamy sand, sand	5.6–6.3	1.1–1.6	417–1026	42506–93317	Rabolle and Spiid (2000)
	Pig manure	NR	NR	83.2/77.6	195	Loke et al. (2002)
	Marine sediment	NR	NR	663, 2590	NR	Smith and Samuelsen (1996)
Tetracycline	Clay loam	NR	1.0	>400	NR	Thurman and Lindsey (2000)
Tetracycline	Clay loam, sandy loam	NR	2.2–4.4	1147–2370	NR	Gupta et al. (2003)
Chlortetracycline	Clay loam, sandy loam	NR		1280–2386		Gupta et al. (2003)
Olaquinox	Pig manure	NR	NR	20.4/9.8	50	Loke et al. (2002)
	Loamy sand, sand	5.6–6.3	1.1–1.6	0.69–1.7	46–116	Rabolle and Spiid (2000)
Efrotomycin	Loam, silt loam, sandy loam, clay loam	5.0–7.5	1.1–4.6	8.3–290	580–11000	Yeager and Halley (1990)
Ciprofloxacin	Sewage sludge	6.5	37	417	1127	Halling-Sørensen (2000)
	Loamy sand	5.3	0.7	427	61000	Nowara et al. (1997)
Enrofloxacin	Clay, loam, loamy sand	4.9–7.5	0.73–1.63	260–5612	16510–99980	Nowara et al. (1997)
Metronidazole	Loamy sand, sand	5.6–6.3	1.1–1.6	0.54–0.67	39–56	Rabolle and Spiid (2000)
Fenbendazole	Silty loam	6.9–7.0	1.6–2.4	0.84–0.91	35–57	Thiele-Bruhn and Leinweber (2000)

NR = not reported;  $K_d$  = soil partition coefficient;  $K_{oc}$  = organic carbon normalized partition coefficient.

<sup>a</sup> pH values were after sorption experiment.



# Estimating Exposure

**Due to the complexity of partitioning and the lack of information on both partitioning and persistence, most are relying on analytical measurements in the environment rather than models**

**Ongoing research is addressing these fate processes to allow for more quantitative modeling in the future**

**Table 1** | Pharmaceuticals detected in surface water monitoring studies

Medicine class	Substances detected	Maximum concentration (ng l <sup>-1</sup> )
Antibiotics	Chloramphenicol	355
	Chlortetracycline	690
	Ciprofloxacin	30
	Lincomycin	730
	Norfloxacin	120
	Oxytetracycline	340
	Roxithromycin	180
	Sulphadimethoxine	60
	Sulphamethazine	220
	Sulphamethizole	130
	Sulphamethoxazole	1,900
	Tetracycline	110
	Trimethoprim	710
Antacid	Tylosin	280
	Cimetidine	580
Analgesic	Ranitidine	10
	Codeine	1,000
	Acetylsalicylic acid	340
	Carbamazepine	1,100
	Diclofenac	1,200
	Aminopyrine	340
	Indomethacine	200
	Ketoprofen	120
	Naproxen	390
	Phenazone	950
Antianginal	Dehydronifedipine	30
Antihypertensive	Diltiazem	49
Antidepressant	Fluoxetine	12
Antihyperlipidemic	Gemfibrozil	790
Antidiabetic	Metformin	150
Antipyretic	Acetaminophen	10,000
Anti-inflammatory	Ibuprofen	3,400
Antiseptic	Triclosan	150
Beta blockers	Betaxolol	28
	Bisoprolol	2,900
	Carazolol	110
	Metoprolol	2,200
	Propanolol	590
	Timolol	10
Bronchodilator	Clenbuterol	50
	Fenoterol	61
	Salbutamol	35
Contraceptive	17a-Ethinylestradiol	4.3
Ectoparasiticides	Cypermethrin	85,100
	Diazinon	580,000
	Emamectin benzoate	1,060
Lipid regulator	Bezafibrate	3,100
	Clofibrate	40
	Gemfibrozil	510
Stimulant	Caffeine	6,000
X-ray contrast media	Diatrizoate	100,000

Data taken from Daughton & Ternes, 1999; Kolpin *et al.*, 2002; Boxall *et al.*, 2004a.

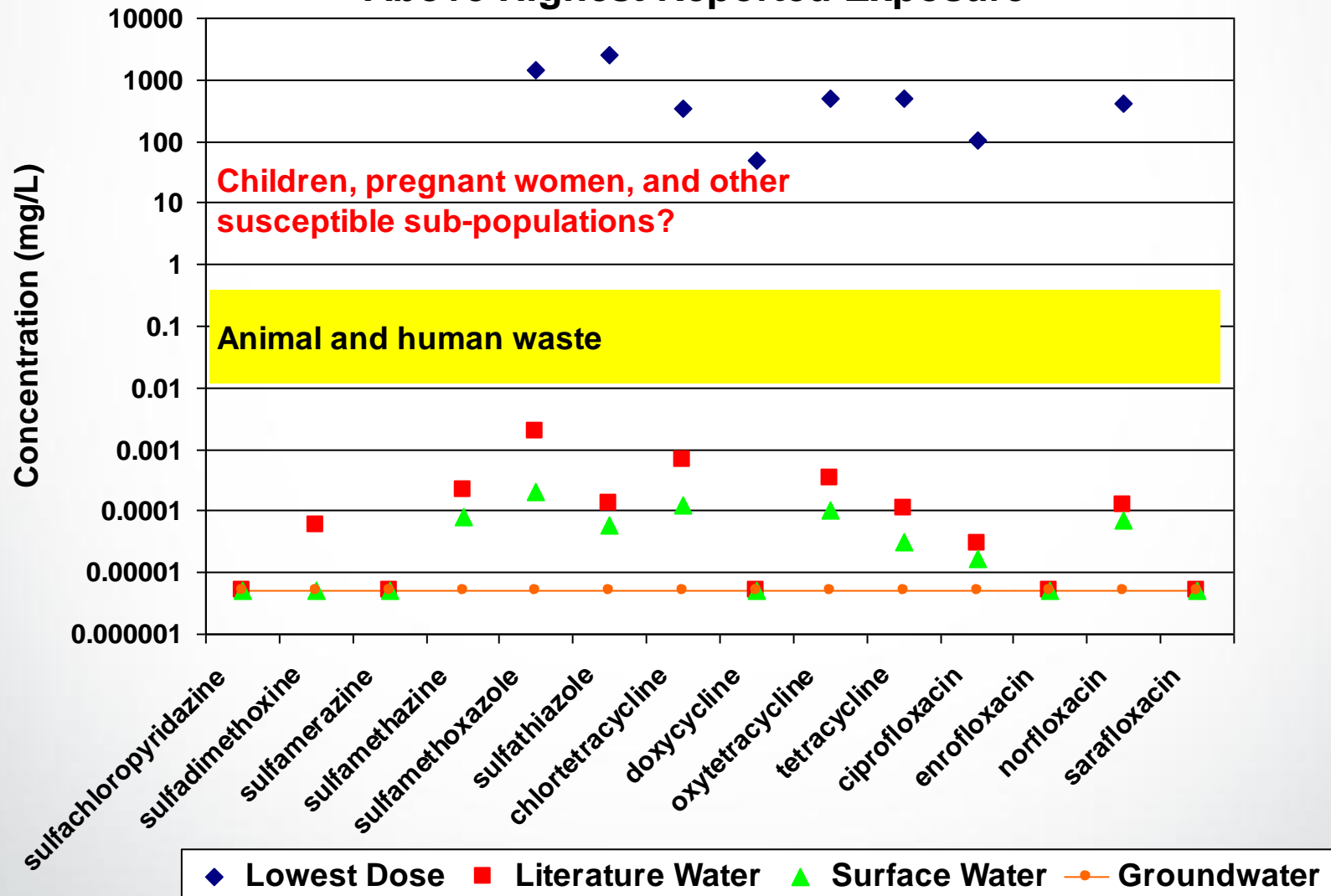
# Environmental Exposure

# Is There Potential for non-AMR Adverse Human Health Effects?

- Substantial information from Phase II toxicity testing, Phase III clinical trials, and subsequent use
- Uncertainty over chronic low-dose toxicity in susceptible populations
- We will use a simple hazard quotient using therapeutic dose as a screen and measured antibiotic concentrations in water from literature and data from recent work of ours

<u>Antibiotic</u>	<u>Dosage Range (mg/kg/d)</u>		<u>Equivalent Drinking Water Dose (mg/L)</u>	
	low	high	low	high
sulfachloropyridazine				
sulfadimethoxine				
sulfamerazine				
sulfamethoxazole	40	100	1400	3500
sulfathiazole	71	250	2485	8750
chlortetracycline	10	30	350	1050
doxycycline	1.4	2.2	49	77
oxytetracycline	14	50	490	1750
tetracycline	14	50	490	1750
ciprofloxacin	2.9	21	101.5	735
enrofloxacin				
norfloxacin	11.4	11.4	399	399
sarafloxacin				

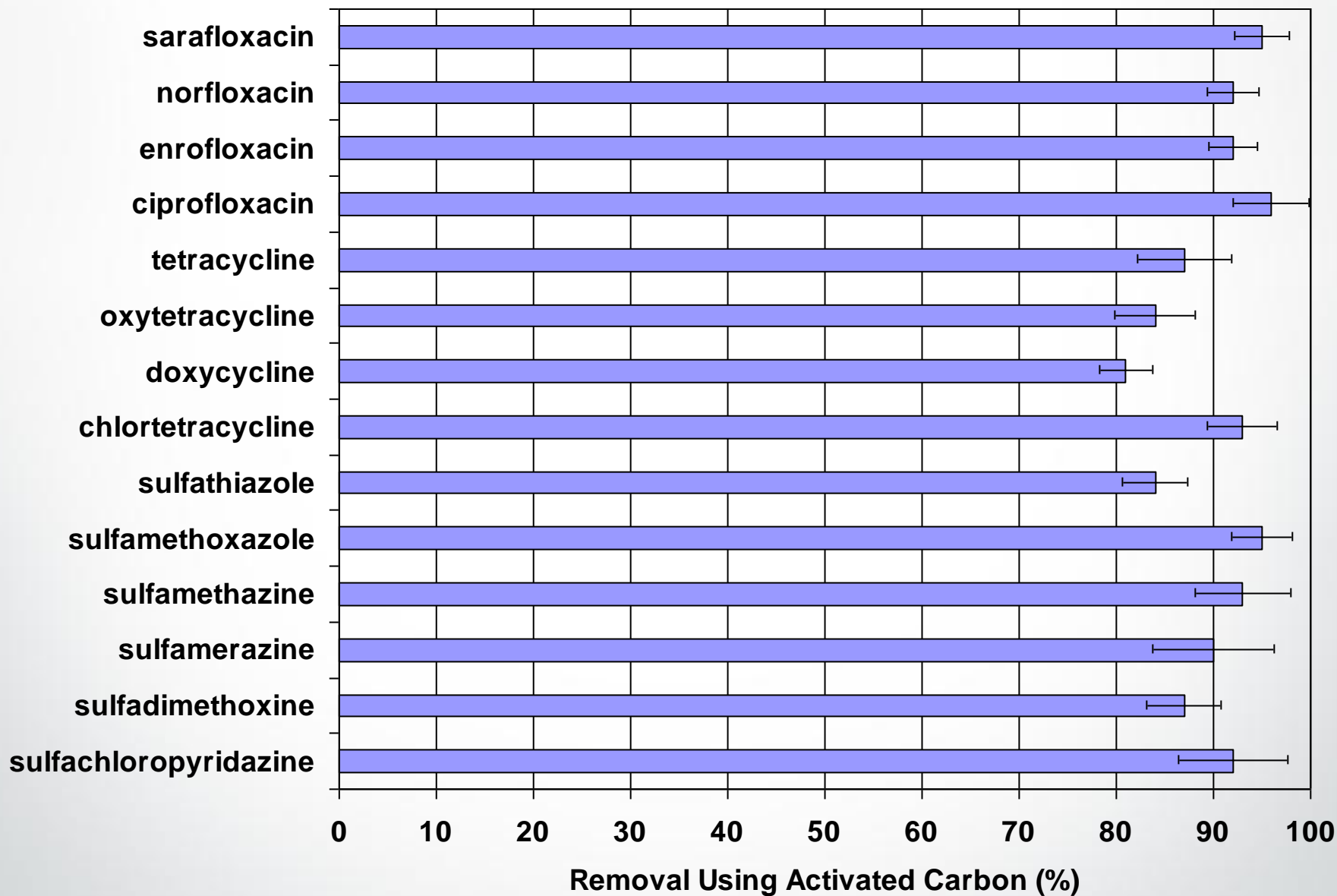
## Therapeutic Dose is One Million Times Above Highest Reported Exposure



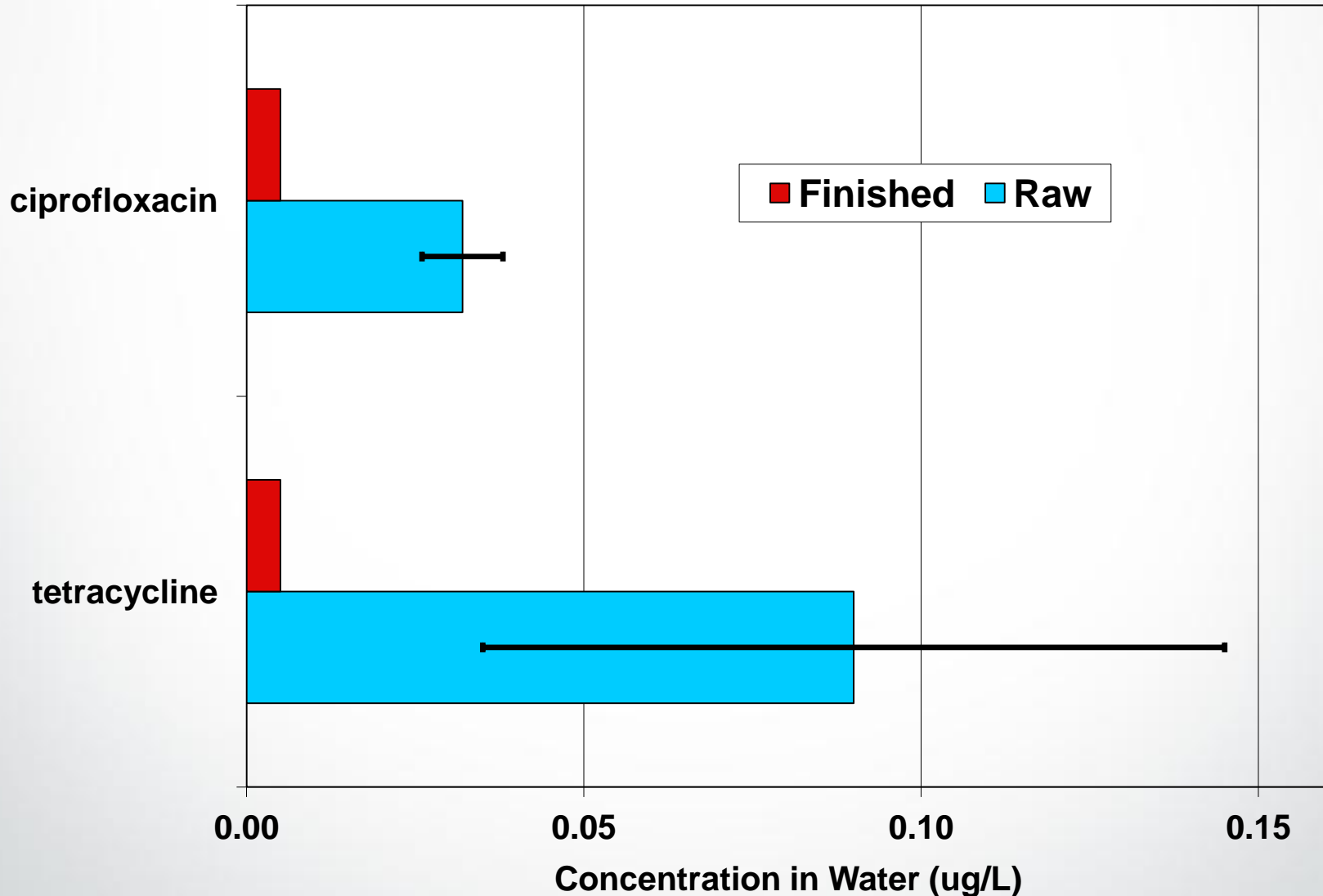
# Are Antibiotics Removed During Drinking Water Treatment?

- **Previous work with other drugs show:**
  - No significant removal with sand (oxic or anoxic)
  - Variable removal rates with flocculation
  - 50-99% removal with ozonation
  - 50-95% removal with granular activated carbon
- **We conducted standard batch adsorption experiments with granular activated carbon to measure removal efficiencies of antibiotics**

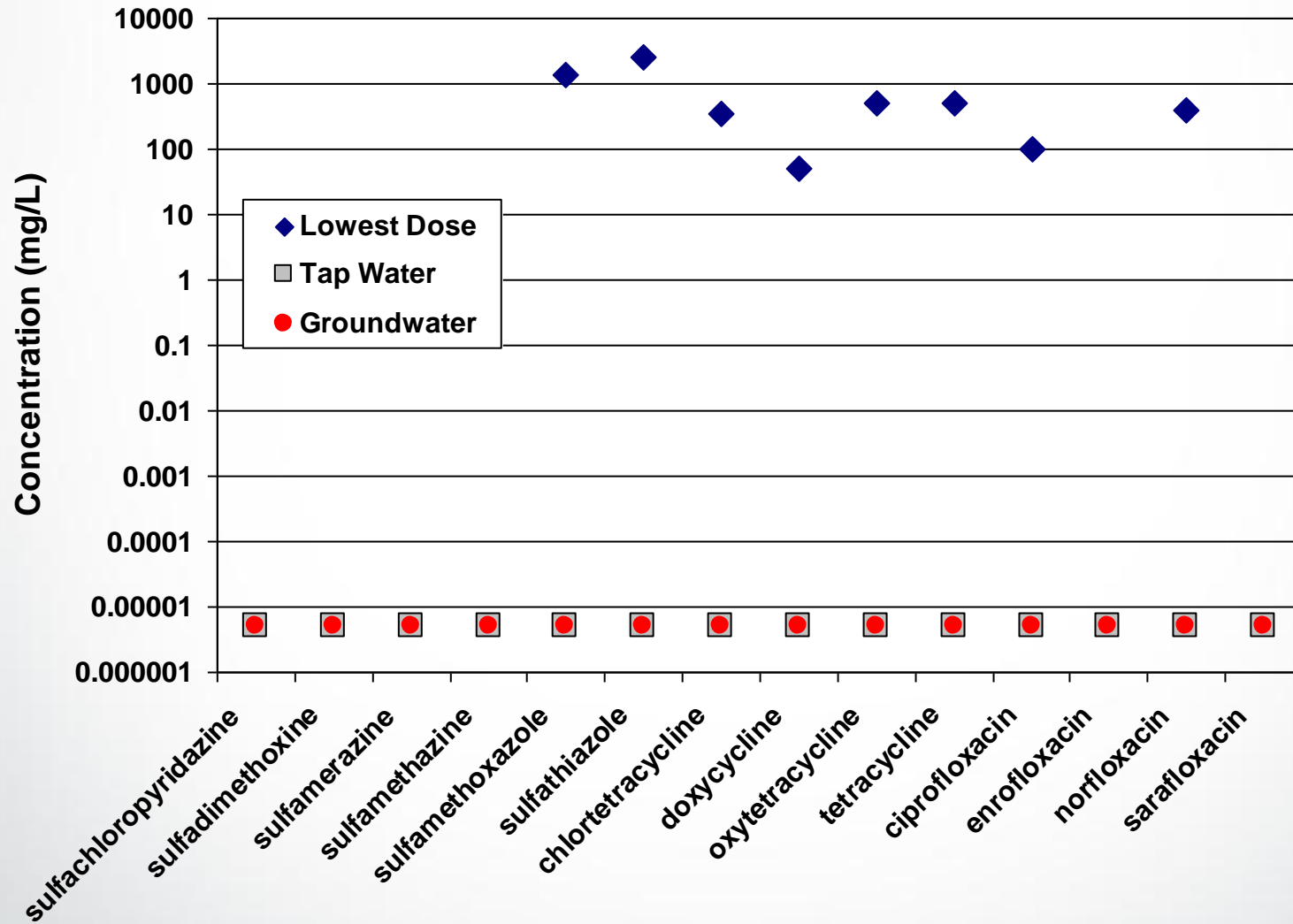




# Removal of Antibiotics in Water Treatment Plant



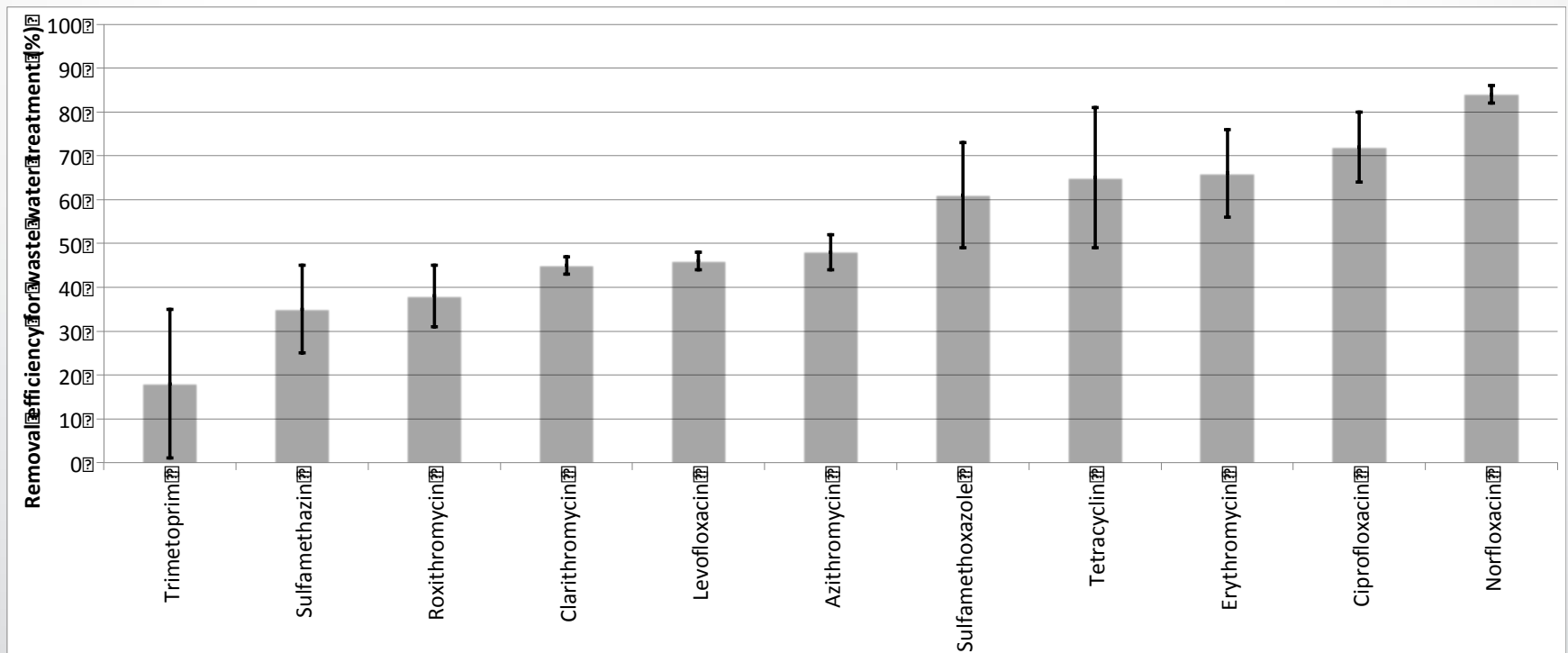
## Antibiotics Were Not Detected in Drinking Water



# Summary of Drinking Water Exposure

- Antibiotics were not detected in drinking water (groundwater beneath municipal sludge/hog waste/manure, or tap water)
- Estimated exposures are  $\sim 10^6$  times below lowest therapeutic dose,
  - susceptible sub-populations not considered, therapeutic dose may slightly overestimate safe exposure for some populations, i.e. no doctor/pharmacist involved
- Activated carbon removes  $\sim 90\%$  of antibiotics
- Additional treatment would remove even more (home)
- We have no evidence of unacceptable human health risk from direct effects using this simple analysis

# Removal of Antibiotics in Sewage Treatment (%)



# Adverse Ecological Effects

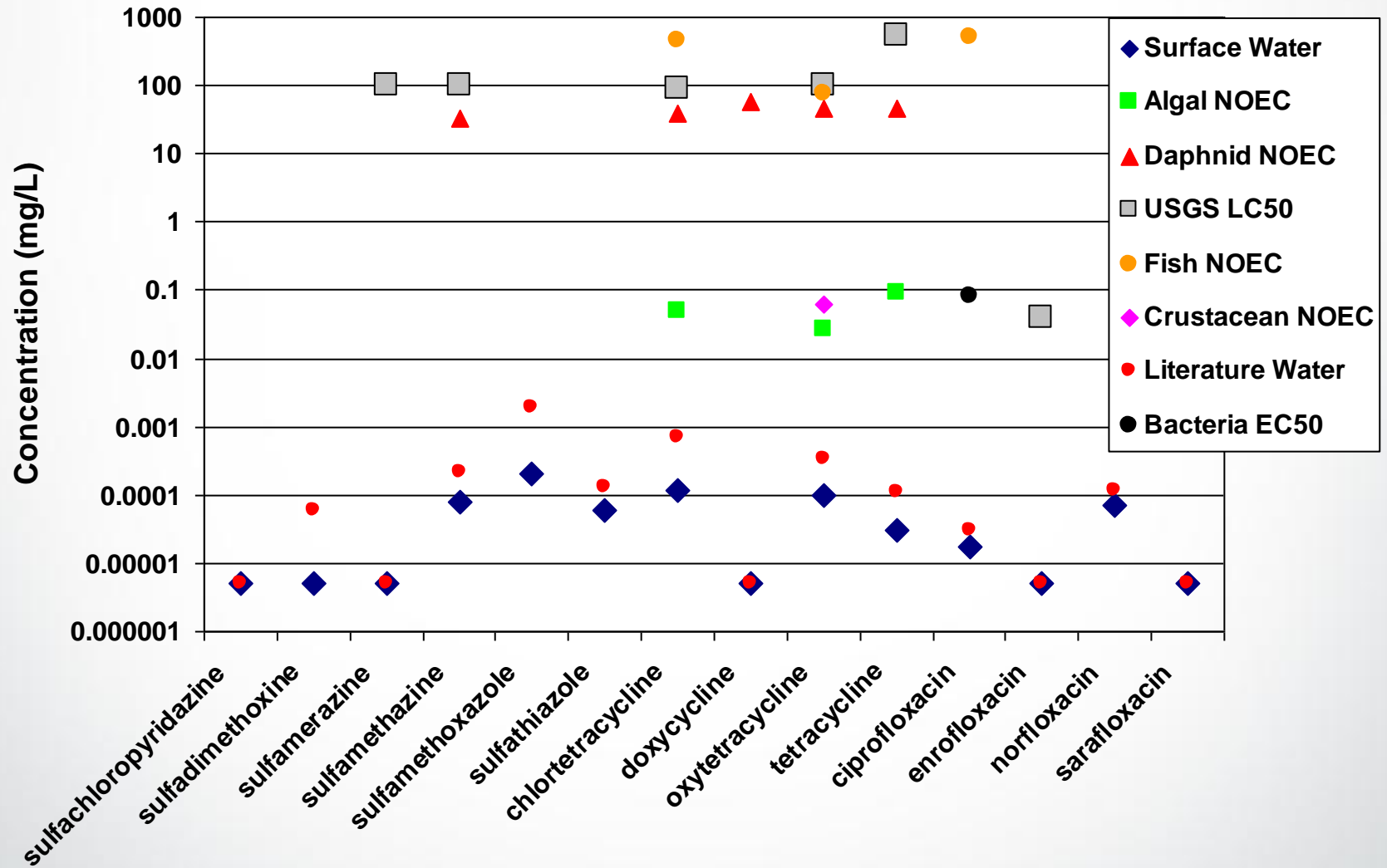
- **Very little information on non-mammalian species**
- **Uncertainty over chronic low-dose toxicity in susceptible populations**
- **We will use hazard quotients and bioassays as screening indicators**
- **Indirect effect of exposure to antibiotic resistant bacteria or changes in microbial populations and food web?**



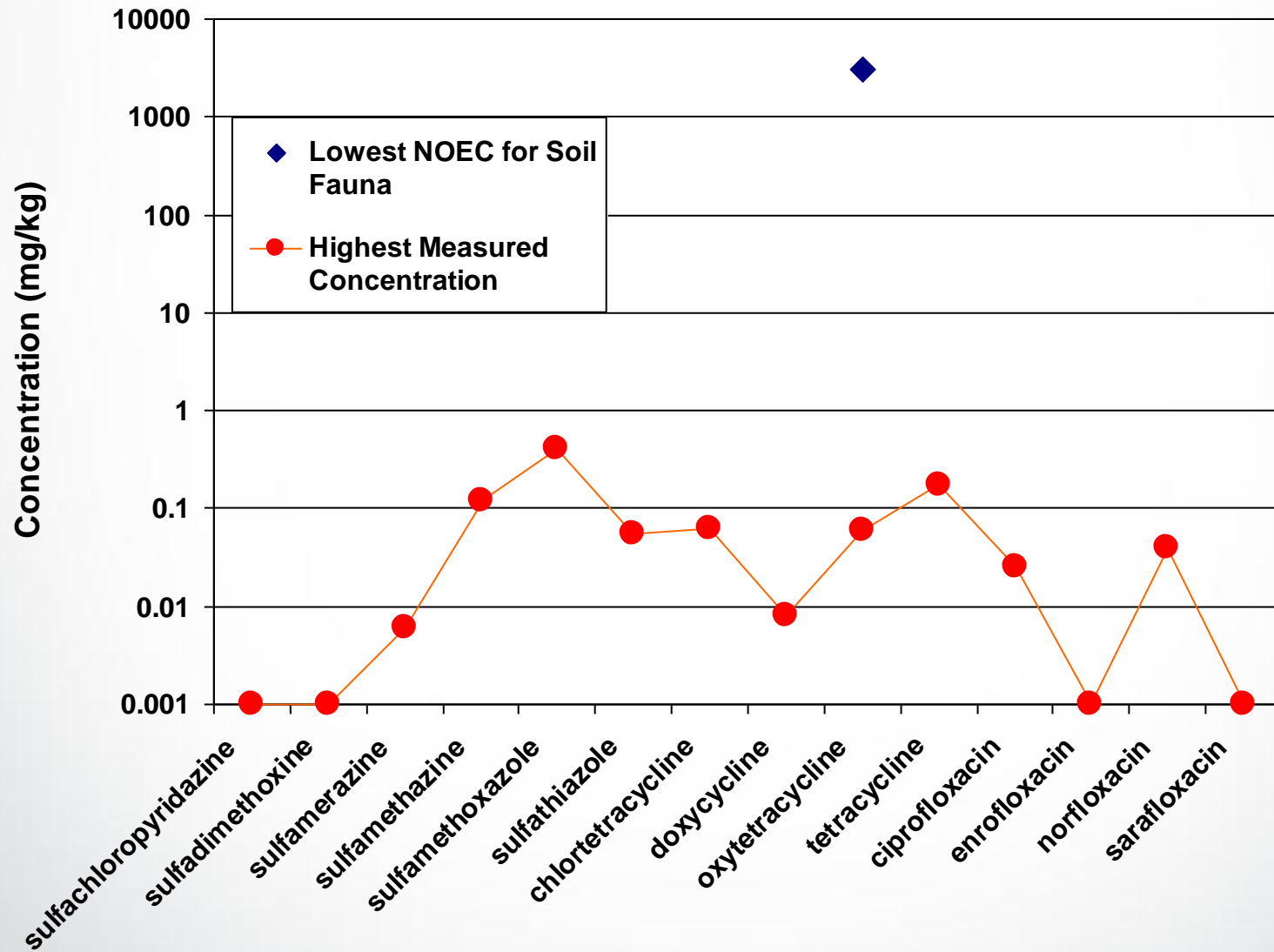
# Indicators of Adverse Ecological Effects

- **Algal toxicity tests (growth inhibition)**
  - *M. aeruginosa* (cyanobacteria) ~ 100 times more sensitive than *S. capricornutum* (green algae)
  - EC50s (mg/L): 0.006 (benzylpenicillin) to > 100
- **Bacteria (*Pseudomonas putida*)**
  - Growth inhibition EC50 = 0.08 mg/L
- **Soil fauna tests**
  - Survival, growth, reproduction, and cocoon hatching success of earthworms, springtails, and enchytraeids (NOEC 2000 to > 5000 mg/kg)
- **Aquatic invertebrates (*Daphnia magna*)**
  - Acute 48-h EC50s (mg/L): 4.6 (oxolinic acid) to > 1000
  - Chronic EC50s (mg/L): 5.4 (tiamulin) to > 250
  - Acute:Chronic ratio ~ 10
- **Fish and crustaceans**

## Antibiotic Exposure Compared to Most Sensitive Effects Level



## Exposure is 50,000 Times Below NOEC in Sludge Ammended Soil



# Bioassays Were Performed at Maximum Aggregate Exposure of all Antibiotics

- *No Adverse Effects Were Observed for All Tests*
- Freshwater and marine tests for:
  - Algal toxicity – no growth inhibition
  - Aquatic invertebrate (*D. magna* and *A. tonsa*)  
no change in survival, growth, reproduction
- No bacterial growth inhibition or resistance tests were performed

# Summary

- Antibiotic residues are detectable in many places and generally follow our expectations of their fate
- Antibiotic fate models provide good generic and evaluative assessments, but the complexity of chemical transformation and partitioning limits their quantitative use
- Both models and measurements indicate low probability for *direct* adverse effects on human and ecological health
- However, we do not yet know the effect of antibiotics in water/sediment/soil on AMR and changes to microbial communities

# Simplified model of the partitioning processes of chemicals

## PSD

P: Parent compound

M: metabolites

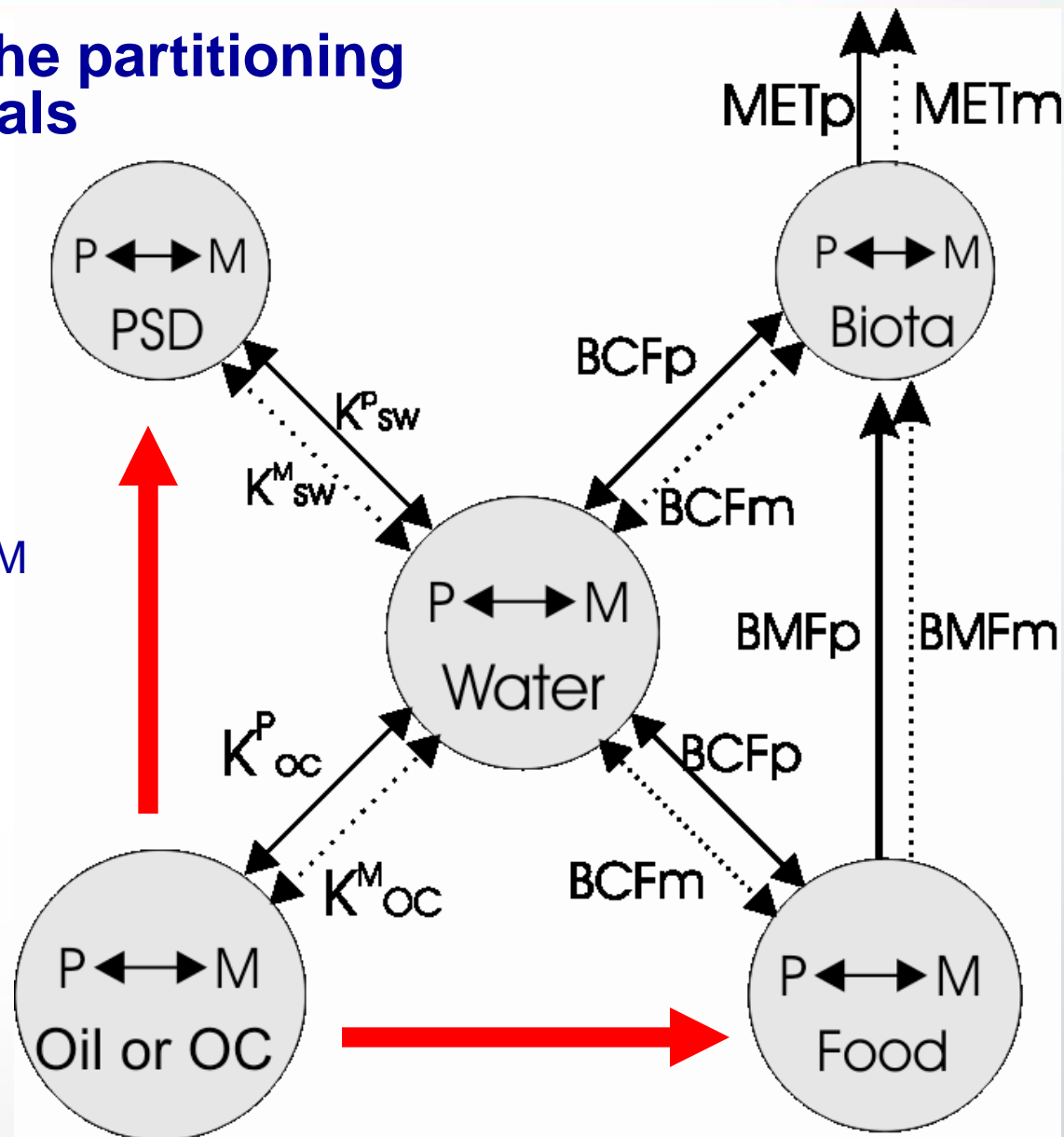
$K_{PSW}$  and  $K_{MSW}$ : PSD-water partition coefficients of P and M

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BMF: biomagnification factor

MET: metabolic clearance



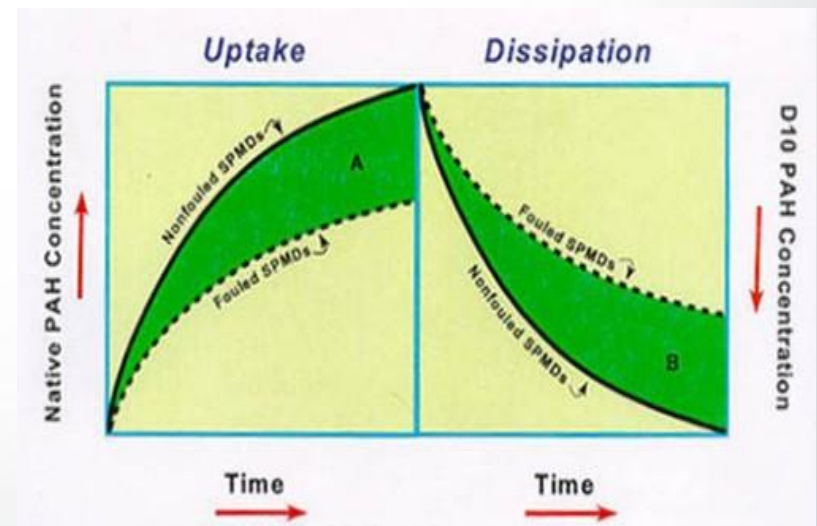


## Passive Sampling Device (PSD): Exposure Dosimeter

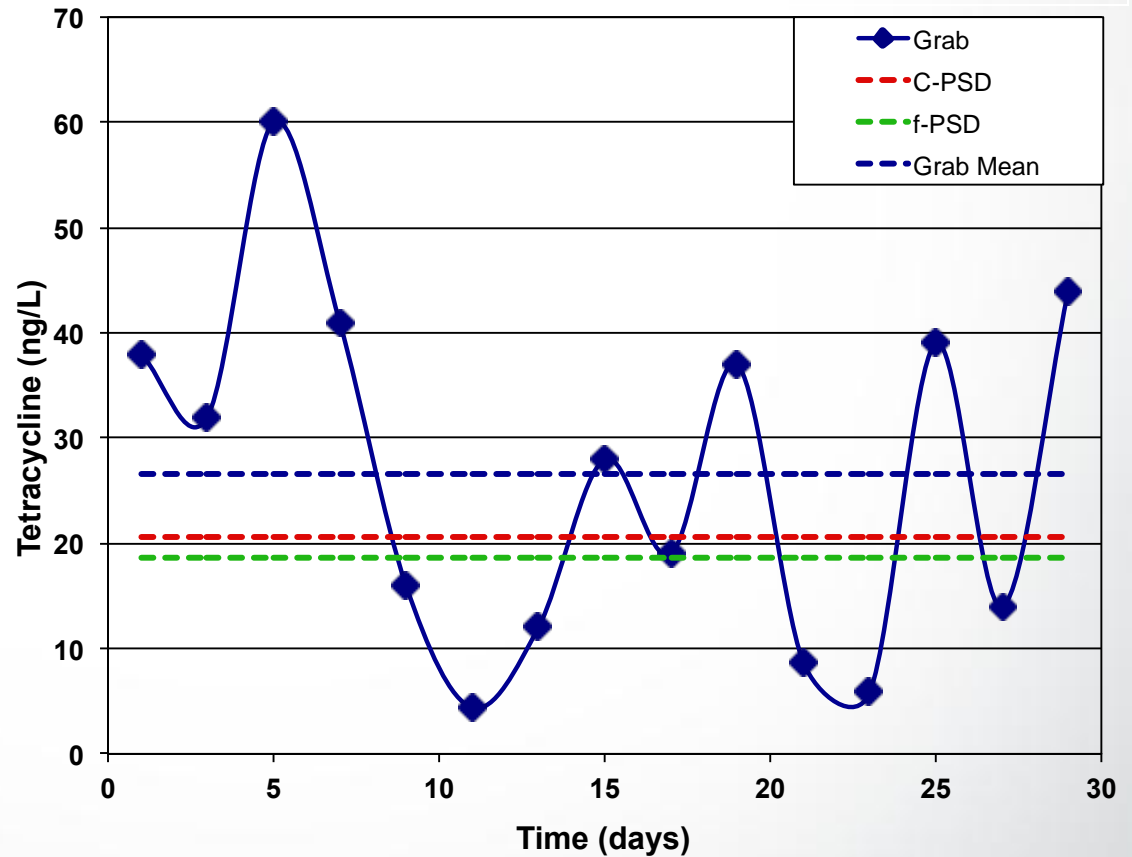
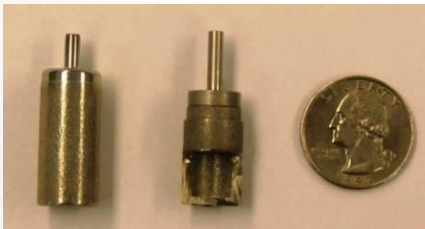
- Sequester and preconcentrate chemicals from water in a time-integrated fashion using polymers (PDMS, PE, POM, etc.)
- Laboratory derived uptake rates ( $R_s$ ) to estimate  $C_w$

$$C_w = N_{\text{PSD}} / R_s * t$$

- Can provide estimate of chronic exposure with lower detection limits and much less cost than traditional grab sampling



# Field Data: Surface Water near CAFO



# Acknowledgments

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